13. (Amended) The process according to claim 1, wherein after elution the proteinaceous material and the solid phase are separated by magnetic beads.

14. (Twice Amended) The process according to claim 1, wherein the isolated proteinaceous material is analyzed by mass spectrometry.

15. (Amended) The process according to claim 10, wherein the magnetic separation is automated.

REMARKS

The Office Action of October 1, 2001 has been received and carefully noted, and the foregoing amended claims and comments set forth below are a complete response thereto.

Claims 1-15 are all the pending claims. By this Amendment, Claim 12 has been canceled without prejudice or disclaimer and Claims 1, 5-7, 10, 13, 14 and 15 have been amended as follows:

Claim 1 is directed to a process for isolating a protein and recites the process steps for doing so;

Claims 5-7 and 14 have been amended to recite proper alternative language;

Claim 10 is directed to the step now occurring between steps (c) and (d) of claim 1;

Claim 13 has been amended to depend from claim 1; and

Claim 15 has been amended to depend from claim 10 and to recite that the magnetic separation is automated.

No new matter has been added, and consideration and entry of the amended claims is requested.

I. Response to Rejection of Claims 1-15 under 35 U.S.C. §112, first paragraph

Claims 1-15 are rejected under 35 U.S.C. §112, first paragraph, for lack of enablement.

A) The Examiner considers the claims enabled for:

-a process of isolation and/or purification of a proteinaceous material comprising the steps of providing an aqueous protein solution,

-contacting the protein solution with a hydrophobic interaction chromatography gel or with magnetically responsive polymer particles coated with hydrophobic agarose particles, and

-separating off other components.

According to the Examiner, the claims as presently written exceed the scope of enablement for this specification, and therefore, in order for one skilled in the art to make or use the invention, undue experimentation is required.

Applicants traverse for the following reasons.

The concept of the invention can be applied to any proteinaceous material. An essential aspect of the invention comprises a combination of hydrophobic groups and hydrophilic groups on its surface. The hydrophobic groups serve to bind the proteinaceous material. The proteinaceous material is preferably bound reversibly and

unspecifically to the hydrophobic groups located on the surface of the solid phase. Binding is not limited by specific binding pairs having high affinity such as streptavidin/avidin, thus, the method of the invention can be used for the isolation and/or purification of any proteinaceous material. The hydrophilic groups on the surface of the solid phase prevent the solid phase from sticking together in an aqueous environment. Combining hydrophilic and hydrophobic groups prevents the particles from agglutinating in aqueous solutions, yet still provides for binding of the proteinaceous material to allow for purification (reversed phase purification, see page 3, lines 14-24).

B) According to the Examiner, the specification does not provide any disclosure regarding the identity of the protein purified, the buffer used, the yield of recovery and the purity of protein after the purification procedure using the solid particles. The specification does not demonstrate how gamma-iron oxide is coated with particles of polysilicic acid or monosaccharide containing hydrophobic groups, the outcome of the purification process using the solid particles with a diameter from 1 nm to 10 mm, or that automation is carried out in one of the purification steps.

The Examiner considers the specification lacking in critical information regarding the preparation of solid particles, the purification conditions, the automation for the purification procedure and the efficiency of the purification procedure.

Applicants traverse for the following reasons.

The specification is enabling for the inventive method with respect to the specific reagents used in performing the method, i.e., the buffer (page 9, lines 2-3) and the elution solution (page 9, lines 9-14). In addition, Example 1 describes the preferred embodiment for the inventive method.

As explained above, the identity of the purified protein (the analyte) is not decisive because the inventive method is a universally applicable method. Therefore, a restriction to a specific embodiment is unduly limiting when the disclosure for the inventive method is fully enabling for one skilled in the art to perform the method.

In view of the foregoing arguments and the enclosed new data, Applicants submit that the claims are fully enabled, and that withdrawal of the rejection is deemed proper.

The Examiner's attention is directed to the additional experimental data enclosed in the attachment, which describes the purity of a protein obtained from the purification procedure of the claimed invention.

II. Response to Rejection of Claims 1-15 under 35 U.S.C. §112, second paragraph

Claims 1-15 are rejected under 35 U.S.C. §112, second paragraph, for indefiniteness.

A) Claims 1-15 are indefinite according to the Examiner because they lack essential steps as follows: the elution of the proteinaceous material from the solid phase and a step whereby the outcome of isolation and/or purification such as yield of recovery and purity of the protein can be determined.

Claims 1-15 now recite a process for isolating the proteinaceous material with the essential steps recited in the claims.

B) Claims 1-15 are indefinite according to the Examiner, for reciting the term "and/or", since it is unclear whether the purification is included in the process.

Applicants have amended the claims to recite "and" or "or" as appropriate.

C) Claims 1-15 are indefinite according to the Examiner, for reciting the phrase "at least one surface" since it is not clear how the solid phase can have many surfaces containing a mixture of hydrophobic and hydrophilic groups.

Applicants submit that the amended claims do not recite "at least one surface", and therefore, the Examiner's rejection is moot.

D) Claim 7 is indefinite according to the Examiner since the claim recites a Markush group and the phrase "and mixtures thereof" is open language in regard to the amounts of each in the mixtures.

Applicants have amended the claim to recite the embodiments using "or" language and to replace the term "mixtures" with "a combination thereof", since the claim language is not intended to imply an amount.

E) Claim 10 recites the limitation "wherein step (c) is performed by magnetic means" in lines 1 and 2, and according to the Examiner, there is insufficient antecedent basis for this limitation in claim 1.

Applicants have amended claim 10 to refer to a step occurring between steps (c) and (d) of Claim 1.

F) Claim 11 is indefinite according to the Examiner since the phrase "at least one washing step" does not indicate the number of washing steps.

Applicants specifically disagree with the Examiner's position, and submit that any number of washings can be performed without affecting the performance of the method or the resultant product.

G) Claim 13 recites the limitation "using magnetic means" in lines 2 and 3, and according to the Examiner, there is insufficient antecedent basis for this limitation in claim 12.

Applicants have amended claim 13 to depend from claim 1 in view of the cancellation of claim 12.

H) Claims 10 and 13 are indefinite since according to the Examiner, the phrase "magnetic means" is undefined.

Applicants have replaced the term with "magnetic separation".

I) Claim 15 is indefinite according to the Examiner, since the phrase "at least one process step is automated" does not indicate how many process steps are intended to be automated.

The specification supports an automated process step for magnetic separation, and claim 15 has been amended to depend from claim 10 since claim 10 recites magnetic separation. Additionally, including this limitation does not raise an issue under §112 since magnetic separation by an automated process was well known in the field of art at the time of filing.

III. Response to Rejection of Claims 1-3, 6, 8, 11, 12 and 14 under 35 U.S.C. §102(b)

Claims 1-3, 6, 8, 11, 12 and 14 are rejected under 35 U.S.C. §102(b) as being anticipated by Belew et al. (J. of Chromatography A, 679 (1994):67-83).

For all the following reasons, the Examiner considers the claims anticipated by Belew. Belew teaches purification of a glycoprotein (recombinant human granulocyte-

macrophage colony-stimulating factor (rhGM-CSF)) from the inclusion bodies produced by transformed E. coli cells by a three-step chromatographic procedure using hydrophobic interaction chromatography (HIC) as the first step. In the HIC, Phenyl Sepharose 6 FF (high sub), which has an average particle size of 90 µm and is made of agarose beads (containing hydroxyl group) derivatized with a phenyl group, is used as packing material for the column. The rhGM-CSF sample is dissolved in ammonium sulfate solution, applied to the HIC column, DNA contaminants are eluted with sodium phosphate-ammonium sulfate solution, and subsequently the bound protein is eluted with sodium phosphate (page 70, left column; page 74; Figure 1), which meets the criteria of claims 1-3, 6, 8, 11 and 12. The purified rhGM-CSF is analyzed by electrospray or laser desorption mass spectrometry (page 72), which meets the criteria of claim 14.

Applicants traverse for the following reasons.

For Belew to be an effective reference under §102(b), it must teach each and every element of the claims, and it must have been published at least one year from the date of filing for the relevant application. As claims 1-3, 6, 8, 11, 12 and 14 are presently written, Belew is not an effective reference against the subject matter.

Below teaches the purification of a glycoprotein (recombinant human granulocyte-macrophage colony-stimulating factor) from inclusion bodies produced by transformed E. coli cells using a three-step chromatographic procedure. The purification process involves hydrophobic interaction chromatography (HIC). The phenyl sepharose 6FF used in the Belew procedure is based on an agarose material derivatized with phenyl groups. Thus, the coating does not include hydroxyl

groups on its surface but only hydrophobic groups. Belew does not teach or suggest combining hydrophilic and hydrophobic groups on the surface of a solid phase.

The purification process of Belew requires no less than three purification steps to achieve sufficient purify of the protein. The present method has an advantage over Belew in that one skilled in the art can purify a proteinaceous material by reversed phase purification using magnetic particle technology.

III. Response to Rejection of Claims 1-3, 6, 7, 8, 11 and 12 under 35 U.S.C. §102(e)

Claims 1-3, 6, 7, 8, 11 and 12 are rejected under 35 U.S.C. §102(e) as being anticipated by Smeds (USPN 6,005,082).

The Examiner considers the claims anticipated in view of Smeds for all of the following reasons. According to the Examiner, Smeds teaches purifying a recombinant protein (coagulation factor VIII) by loading an aqueous protein solution onto a hydrophobic interaction chromatography gel, which has aromatic or aliphatic group such as octyl or butyl on agarose matrices. For example, Butyl Sepharose 4 FF, which has an average particle size of 90 μm and is made of agarose beads (containing hydroxyl group) derivatized with butyl group, is used as packing material for the column. The factor VIII solution is loaded onto the HIC column for adsorbing factor VIII to the gel surface, and the bound factor VIII is then eluted with buffer (column 4, line 49 to column 5, line 37; column 6, lines 52-65; Examples), which meets the criteria of claims 1-3, 6, 7, 8, 11 and 12.

Applicants traverse for the following reasons.

For Smeds to be an effective reference under §102(e), it must be by another, it must teach each and every element of the claims, and it must have been described in a U.S. patent having an effective U.S. filing date before applicant's date of invention. As claims 1-3, 6, 8, 11, 12 and 14 are presently written, Belew is not an effective reference against the subject matter.

Smeds describes purification of factor VIII by loading an aqueous solution containing factor VIII onto a hydrophobic interaction chromatography gel which has aromatic or aliphatic groups on agarose matrices. Smeds also describes a hydrophilic starting material, wherein hydrophilic groups such as hydroxyl groups, are derivatized with hydrophobic groups so that a surface is formed having only hydrophobic groups, and thus, no longer represents a mixed surface of hydrophilic and hydrophobic groups.

Accordingly, Smeds teaches that at least one surfactant has to be present in the aqueous solution and/or a buffer solution used to equilibrate the gel (column 4, lines 12-14). In contrast thereto, the isolation method of the invention is based on the combination of hydrophilic and hydrophobic groups being present on the surface of the solid phase, and the surfactant is not required.

Another essential aspect of Smeds is the use of a column where the particles are in close contact to obtain what Smeds refers to as "deliberate agglutination ". The particles according to Smed do not possess hydrophilic groups on their surface, and as for the column of Smeds there is no need to prevent agglutination of the particles. In contrast, agglutination is avoided in the case of the method of the invention, and thus, handling of the particles having reversed phase affinity in solution is novel. One essential aspect of the present invention is the prevention of agglutination of

particles for reversed phase purification, e.g. of peptides or proteins, in aqueous solution by combining hydrophilic and hydrophobic groups or areas on the surface of solid phases.

For all of the foregoing reasons, Applicants submit that the claims do not read on, and therefore, are not anticipated by Smeds.

IV. Response to Rejection of Claims 1-14 under 35 U.S.C. §103(a)

Claims 1-14 are rejected under 35 U.S.C. §103(a) as being obvious over Wang and Belew or Smeds.

The Examiner considers the claims *prima facia* obvious in view of Wang and Belew or Smeds for all of the following reasons. The Examiner cites Wang for teaching magnetically responsive fluorescent polymer particles comprising polymeric core particles (1-100 microns) coated evenly with a layer of polymer containing magnetically responsive metal oxide and the surface of magnetically responsive polymer particles can be coated further with another layer of functionalized polymer and used as solid phase for affinity purification.

Since, according to the Examiner, Wang is silent with respect to using magnetic polymer particles further coated with particles comprising a mixture of hydrophobic groups and hydrophilic groups and a process for purification proteinaceous materials, the Examiner cites Belew for teaching a glycoprotein purified by hydrophobic interaction chromatography (HIC) using a Phenyl Sepharose 6 FF (high sub) gel (page 70, left column; page 74; Figure 1), and Smeds for teaching a recombinant protein purified by

loading an aqueous solution onto a hydrophobic interaction chromatography gel having octyl or butyl group on agarose matrices.

Applicants traverse for the following reasons.

Wang describes magnetically responsive fluorescent polymer particles comprising polymeric core particles coated evenly with a layer of polymer containing magnetically responsive metal oxide. The surface of these magnetically responsive polymer particles can be coated with another layer of functionalized polymer and can be used as solid phase for affinity purification

Wang does not teach magnetic polymer particles coated with particles comprising a mixture of hydrophobic groups and hydrophilic groups. Nor does Wang teach a process for the purification of proteinaceous material. Wang describes that the magnetically responsive polymer particles can be used on very specific bonding of biological materials. Affinity purification is well known in the art and Wang describes such a method involving an interaction between a specific ligand and a substance of interest. Wang's purification process is limited to a specific ligand and its respective binding substance, thus Wang's method teaches away from an object of the present invention, namely a purification process using reversed phase chromatography based on nonspecific reversible binding (page 2, lines 2-3). In addition, neither reversed phase chromatography nor a combination of reversed phase purification with other separation technologies such as magnetic particle technology, are described anywhere in the Wang reference.

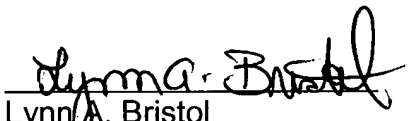
In view of all of the foregoing reasons, Applicants submit that the claims are nonobvious over the references alone or in combination, and that withdrawal of the rejection is deemed proper.

CONCLUSION

In view of the foregoing amended claims and arguments for patentability of the claims, Applicants submit that the Examiner's rejection of the claims under 35 U.S.C. §§102(b), 102(e), 103(a) and 112, first and second paragraphs, have been met and overcome. Applicants request that the Examiner allow the application to pass to issuance.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,



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MARKED-UP COPY OF AMENDED CLAIMS

1. (Amended) A process for [the isolation and/or purification determining of] isolating a proteinaceous material in an aqueous sample comprising the steps:

(a) providing [an] the aqueous sample comprising [a] the proteinaceous material,

(b) contacting the aqueous sample with a solid phase [having a surface comprising a mixture of hydrophobic groups and hydrophilic groups on at least one surface thereof, wherein] for binding [said] the proteinaceous material [binds] to the [at least one surface] solid phase, [and]

(c) [separating off other sample] removing unbound components from the solid phase of (b), and

(d) thereafter, isolating the proteinaceous material by eluting the proteinaceous material from the solid phase.

5. (Amended) The process of claim 4, wherein the particles are paramagnetic [or/and] or ferromagnetic.

6. (Twice Amended) The process according to claim 1, wherein the hydrophobic groups are [selected from] alkyl groups [and/or] or aryl groups.

7. (Amended) The process according to claim 6, wherein the alkyl groups are at least one of [selected from] a C_8 alkyl[,] and a C_{18} alkyl [and mixtures thereof].

10. (Amended) The process according to claim 1, wherein [step] between steps (c) and (d) , the solid phase having bound proteinaceous material, is [performed] separated by magnetic [means] separation.

13. (Amended) The process according to claim [12] 1, wherein after elution the proteinaceous material and the solid phase are separated [using] by magnetic beads.

14. (Twice Amended) The process according to claim 1, wherein the isolated [and/or purified] proteinaceous material is analyzed by mass spectrometry.

15. (Amended) The process according to claim [1] 10, wherein [at least one process step] the magnetic separation is automated.